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Gender-specific reduction of hepatic Mrp2 expression by high-fat diet protects female mice from ANIT toxicity

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Abstract

Emerging evidence suggest that feeding a high-fat diet (HFD) to rodents affects the expression of genes involved in drug transport. However, gender-specific effects of HFD on drug transport are not known. The multidrug resistance-associated protein 2 (Mrp2, Abcc2) is a transporter highly expressed in the hepatocyte canalicular membrane and is important for biliary excretion of glutathione-conjugated chemicals. The current study showed that hepatic Mrp2 expression was reduced by HFD feeding only in female, but not male, C57BL/6J mice. In order to determine whether down-regulation of Mrp2 in female mice altered chemical disposition and toxicity, the biliary excretion and hepatotoxicity of the Mrp2 substrate, α -naphthylisothiocyanate (ANIT), was assessed in male and female mice fed control diet or HFD for 4 weeks. ANIT-induced biliary injury is a commonly used model of experimental cholestasis and has been shown to be dependent upon Mrp2-mediated efflux of an ANIT glutathione conjugate that selectively injures biliary epithelial cells. Interestingly, HFD feeding significantly reduced early-phase biliary ANIT excretion in female mice and largely protected against ANIT-induced liver injury. In summary, the current study showed that, at least in mice, HFD feeding can differentially regulate Mrp2 expression and function and depending upon the chemical exposure may enhance or reduce susceptibility to toxicity. Taken together, these data provide a novel interaction between diet and gender in regulating hepatobiliary excretion and susceptibility to injury.

Keywords

ANIT; gender; high-fat diet; Mrp2; cholestasis; hepatotoxicity

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Introduction

In humans, emerging evidence show high-fat diet (HFD) and obesity affects biliary excretion and chemical disposition; however, the underlying mechanism is not clear (Hanley *et al.*, 2010). In rodents, studies have demonstrated that HFD leads to obesity, which is associated with altered expression of genes involved in biliary drug excretion (Bowes and Renwick, 1986; Lickteig *et al.*, 2007). Most of the studies were performed in male animals and the effects of gender on the expression of drug transporters after HFD feeding are not known.

The multidrug resistance-associated protein 2 (Mrp2, Abcc2) is a canalicular efflux transporter highly expressed in hepatocytes and is critical for the biliary excretion of glutathione and glutathione-conjugated organic anions (Jansen and Peters, 1985; Huber *et al.*, 1987; Dietrich *et al.*, 2001). Specific mutations in the human *ABCC2* gene create non-functional MRP2 protein resulting in Dubin–Johnson syndrome (Wada *et al.*, 1998). Patients with Dubin-Johnson syndrome have chronic hyperbilirubinemia due to impaired biliary excretion of conjugated bilirubin. In addition to gene mutations, the expression of Mrp2 is subject to various transcriptional and post-transcriptional modifications (Gerk and Vore, 2002). For example, the expression of Mrp2 mRNA is induced by activation of pregnane X receptor (PXR), constitutive androstane receptor (CAR) and farnesoid X receptor (FXR) via binding of the nuclear receptors to an everted repeat separated by 8 nucleotide (ER8) sequence in the *Abcc2* gene promoter (Kast *et al.*, 2002). In contrast, the expression of Mrp2 mRNA and protein has been shown to be reduced by endotoxin or cholestasis (Trauner *et al.*, 1997; Vos *et al.*, 1998), as well as by activation of the peroxisome proliferator-activated receptor α (PPAR α) (Trauner *et al.*, 1997; Johnson and Klaassen, 2002).

Furthermore, the hepatic expression of Mrp2 demonstrates gender dimorphism. Under normal diet, the expression of Mrp2 is higher in female mice than in male mice (Maher *et al.*, 2005; Rost *et al.*, 2005; Suzuki *et al.*, 2006; Lu and Klaassen, 2008). However, treatment with ethinylestradiol reduced Mrp2 protein but not mRNA levels, indicating post-transcriptional modification in regulating Mrp2 levels (Trauner *et al.*, 1997). The effect of HFD and obesity on the expression of the Mrp2 gene in rodents has been reported but the results were controversial. The mRNA levels of Mrp2 have been shown to be reduced in the livers of male rats with HFD (Lickteig *et al.*, 2007). However, genetically-induced obesity (ob/ob mice) does not alter the mRNA expression of Mrp2, but increased protein levels in both male and female mice (Cheng *et al.*, 2008).

Because Mrp2 is critical for biliary excretion of chemicals and HFD is a commonly recognized etiology for metabolic syndrome, clarification of the effect of HFD on Mrp2 expression in both genders contributes to an in-depth understanding of the disposition of drugs which are substrates of this transporter. In the current study, we have determined the effects of HFD feeding on Mrp2 mRNA and protein expression in both male and female C57BL/6 mice. The functional relevance of gender-divergent responses to HFD was assessed by examining biliary excretion and hepatic toxicity of α -naphthylisothiocyanate (ANIT). A glutathione conjugate of ANIT is a substrate of Mrp2 that elicits hepatotoxicity by specifically disrupting bile duct epithelial cells (Goldfarb *et al.*, 1962; Dietrich *et al.*, 2001). Because of bile duct damage, bile acids accumulate in livers of ANIT-treated mice leading to hepatocyte injury and inflammation.

Materials and Methods

Materials and diet

ANIT was obtained from Sigma Inc. (St. Louis, MO). The HFD was purchased from Bioserv Inc. (Frenchtown, NJ), which contains 0.15% cholesterol, 34% saturated fatty acids, 50% mono-unsaturated fatty acids, and 16% polyunsaturated fatty acids. A normal chow diet (control diet, CD) was from Harlan Teklad (Rodent Diet #8604), containing 24% minimum protein and 4% fat. All other chemicals, unless otherwise indicated, were obtained from Sigma.

Animal treatment and tissue collection

At 8- to 12-weeks old, male and female C57BL/6 mice (n=5 mice per group) were fed either a control diet (CD) or the HFD for 4 weeks, with food intake and body weight monitored weekly. At the end of the feeding, a single dose of ANIT (75 mg/kg), which was dissolved in saline, was administered to non-fasted mice by oral gavage (10 ml/kg). Thirty-six hours later, blood was collected by retro-orbital bleeding for the separation of serum. Livers were collected and divided into 3 parts, with one part snap frozen in liquid nitrogen for mRNA and protein expression analysis, one part frozen in the OCT compound for myeloperoxidase (MPO) and Mrp2 staining, and one part fixed in 10% PBS-buffered formalin and subsequently embedded in paraffin for hematoxylin and eosin (H&E) staining.

Liver histopathology and neutrophil quantification

Formalin-fixed livers were cut into 5- μ m sections and stained for H&E for histopathologic analysis. Frozen livers embedded in OCT compound were sectioned at 5 μ m and stained with MPO antibody using a standard procedure. To quantify neutrophil infiltration, neutrophils were counted in 20 randomly chosen fields with $\times 400$ magnification of each liver specimen stained for MPO.

Serum parameter analysis

Kits for analyzing serum activity of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin were purchased from Pointe Scientific, Inc. (Canton, MI). Kit for measuring serum levels of total bile acids was purchased from Wako Inc (Richmont, VA). All measurements were performed according to the manufacturers' instructions.

Quantification of mRNA expression

Total RNAs were isolated with the Trizol reagent purchased from Invitrogen (Carlsbad, CA) according to the manufacturer's instructions. The integrity of total RNA was confirmed by the MOPS gel electrophoresis. The concentration of the total RNA was determined by spectrophotometry. The cDNAs were generated using random primers and Script-II reverse transcriptase (Invitrogen). The mRNA expression was quantified using the Sybr green chemistry with a standard protocol and the expression level was normalized to 18s RNA levels. The primer sequences used for real-time quantitative PCR (qPCR) can be obtained upon request.

Western blot

Livers were homogenized in sucrose-Tris buffer (0.25M sucrose, 10 mM Tris-HCl, pH 7.4) containing protease inhibitors. Protein concentrations were determined using Pierce protein assay reagents according to the manufacturer's recommendations (Pierce Biotechnology, Rockford, IL). Homogenates were loaded (without boiling) and proteins were separated on 5% Tris-HCl SDS-polyacrylamide gels (Bio-rad, Hercules, CA). Proteins were transferred overnight at 4°C to polyvinylidenedifluoride membranes. Membranes were blocked for 2

hours in blocking buffer (5% non-fat dry milk with 0.05% Tween 20). Primary (Mrp2, M₂III-5) antibody was diluted 1:600 in blocking buffer (Aleksunes *et al.*, 2006). The M₂III-5 antibody was provided by Dr. George Scheffer (VU Medical Center, Amsterdam, The Netherlands). Blots were subsequently incubated with an anti-mouse horseradish peroxidaseconjugated secondary antibody for 1 hour. Protein-antibody complexes were detected using an ECL chemiluminescent kit (Pierce Biotechnology, Rockford, IL) and exposed to Fuji Medical X-ray film (Fisher Scientific, Springfield, NJ).

Indirect immunofluorescence analysis

Immunostaining for Mrp2 was performed on frozen liver sections (5 μ m) using a Mrp2 antibody from Dr. Bruno Stieger (University Hospital, Zurich, Switzerland) as described previously (Maher *et al.*, 2007). Briefly, liver cryosections were fixed with 4% paraformaldehyde for 5 minutes. All antibody solutions were filtered through 0.22 μ m membrane syringe-driven filter units. Liver sections were blocked with 5% serum/ phosphate-buffered saline with 0.1% Triton X-100 for 1 hour, and then incubated with primary antibody diluted 1:100 in blocking buffer for 2 hours at room temperature. Sections were subsequently washed, and incubated for 1 hour with goat anti-rabbit Alexa 488 IgG (Invitrogen, Grand Island, NY). Images were captured on an Olympus BX41 fluorescence microscope with a DP70 camera and DP Controller software (Olympus America Inc., Center Valley, PA) at 200x magnification. Negative controls without primary antibody were included in the analysis (data not shown). All sections were both stained and imaged under uniform conditions.

Plasma elimination and biliary excretion studies

Mice were anesthetized by injection of ketamine/midazolam (100 mg/kg and 5 mg/kg, respectively, *i.p.*), and body temperatures were maintained at 37°C by rectal probe-controlled heating pads. Subsequently, the right carotid artery was cannulated with PE-10 tubing, and the common bile duct was cannulated with the shaft of a 30-gauge needle attached to PE-10 tubing through a high abdominal incision. Depth of anesthesia was monitored by pinching the footpad before and throughout the surgery, and if necessary, extra anesthetic drug was administered during period of sample collection. After the initial 10-minute bile collection, ANIT (135 μ mol/kg) was injected into the right femoral vein (10 ml/kg). ANIT was dissolved in a vehicle consisting of 5% ethanol, 5% cremophor EL, and 90% sterile saline according to Hu and Morris (2005). After ANIT injection, bile samples were collected in 15-minute periods into pre-weighed 0.6 ml microcentrifuge tubes for 4 periods after the 10-minute pre-bile collection. The tubes into which the bile was collected were immersed into ice. Volumes of bile samples were measured gravimetrically, using 1.0 for specific gravity. Thirty to thirty five μ l of blood was collected into heparinized PCR tubes at 2, 7.5, 22.5, 37.5 and 60 minutes after ANIT injection.

Detection of ANIT by reverse-phase HPLC method with UV detection

The HPLCUV detection of ANIT was performed according to a described method (Hu and Morris, 2003). Plasma or bile samples were mixed with acetonitrile at a ratio of 1 sample: 9 acetonitrile, and were vortexed and centrifuged at 4°C at 15,000 $\times g$ for 10 minutes. Supernatant was spiked with 100 μ M of naphthalene (NE) as an internal standard. Then the samples were analyzed with a Waters e2695 HPLC with UV detection at 305 nm. The reversed-phase chromatography was performed with an xBridge C₁₈ 3.5 μ m column 4.6 $\times$ 150 mm I.D. (Waters, MA), and eluted isocratically with a mobile phase consisting of acetonitrile/H₂O (70:30, v/v). The Empower II software (Waters) was used for instrument control and data analysis.

Pharmacokinetics

The plasma concentrations (C_p) for ANIT were found to fit an open 2-compartment pharmacokinetic model described by the biexponential equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

where A and α hybrid constants are, respectively, the y-intercept and elimination rate constant of the distributive phase, and B and β hybrid constants describe the y intercept and elimination rate constant of the terminal phase of the 2 components of the curve. The data were fitted to the exponential components of the equation by a method of least squares with the coefficient of correlation used as the indicator of data fit. This curve fitting was made by Sigma plot 10.0 ($R^2 > 0.99$; Systat Software Inc., San Jose, CA). The model describes the distribution of ANIT between the dose of ANIT (D), a central compartment, $V_{d\text{cent}}$ (plasma and plasma-like tissue), and a peripheral compartment ($V_{d\text{perif}}$ - all other tissues that behave kinetically differently from plasma). The distribution half-life time ($T_{1/2\text{dist}}$), elimination half-life time ($T_{1/2\text{el}}$), the apparent volume of distribution at steady state (V_{app}) for the central compartment (V_{cent}) and the peripheral compartment (V_{perif}), and total body clearance (Cl) were calculated based on the following equations:

$$T_{1/2\text{dist}} = \frac{0.693}{\alpha}$$

$$T_{1/2\text{el}} = \frac{0.693}{\beta}$$

$$V_{d\text{cent}} = \frac{D}{A+B}$$

$$V_{d\text{perif}} = \frac{D}{B}$$

$$V_{d\text{app}} = \frac{D}{A/\alpha + B/\beta}$$

$$Cl = V_{d\text{app}} \frac{0.693}{T_{1/2\text{el}}}$$

Statistical analysis

The data are expressed as mean $\pm$ SE. For pharmacokinetic data, the individual values were log-transformed to obtain a normal data distribution, which was required for the paired standard t-tests. The differences between the CD- and HFD-fed mice, or the difference between male and female mice, were determined by t-test. The statistical significance

between multiple groups was analyzed by one-way ANOVA followed by the Student-Newman-Keuls test. P values of <0.05 were considered significant.

Results

Effect of HFD on hepatic Mrp2 expression in female and male mice

The livers of CD- and HFD-fed male and female mice were examined for mRNA expression of genes involved in biliary excretion and bile acid homeostasis. Overall, the expression of transporters and enzymes were changed by HFD feeding in a similar fashion in both genders (Table 1). However, a significant gender difference in Mrp2 expression was observed. On CD, female mice expressed slightly higher levels of liver Mrp2 mRNA than male mice. When fed the HFD for 4 weeks, female mice showed a 43% reduction of hepatic Mrp2 mRNA levels compared to CD-fed female mice that was not observed in male mice (Figure 1A). The basal protein levels of Mrp2 under CD diet were slightly higher in female than in male mouse livers. Consistent with mRNA levels, the hepatic expression of Mrp2 protein tended to increase and decrease after HFD feeding in male and female mice, respectively, although the data were not statistically significant (Figure 1B). Immunofluorescent staining of liver sections confirmed canalicular staining of Mrp2 protein levels and demonstrated reduced staining intensity of Mrp2 protein in livers of female mice after HFD feeding (Figure 1C).

Plasma elimination of ANIT in female and male mice following HFD feeding

Mrp2 mediates the biliary excretion of many glutathione-conjugated chemicals. To verify whether reduced Mrp2 expression affects biliary excretion of ANIT, the compound was administered to anesthetized mice of both genders with or without HFD. ANIT-glutathione is a *bona fide* Mrp2 substrate revealed by a study showing that Mrp2-deficient rats were completely protected from ANIT-induced cholestasis and liver injury, as mentioned earlier. Figure 2 illustrates the plasma elimination of ANIT following i.v. administration of ANIT at 135 $\mu\text{mol/kg}$. This dose was chosen based on the previous work of Hu and Morris (Hu and Morris, 2005). The plasma disappearance curves indicate that ANIT can be described by a two-compartment open model of elimination. Shown in Table 2, the major pharmacokinetic parameters were calculated and there were no differences of the plasma ANIT concentration between HFD- and CD-fed male mice. In contrast, the plasma concentration of ANIT was 3-fold higher in female mice fed the HFD than those fed the CD. In addition, while the half-lives were similar, the V_d and Cl were 70% lower in HFD-fed female mice than in male mice.

Effects of HFD on bile flow and biliary excretion of ANIT in both genders

Compared to the CD, the HFD did not change the bile flow/kg of body weight in either gender (Figure 3A), nor the bile flow/g liver in male mice (Figure 3B), but decreased the bile flow/g liver in female mice by approximately 50 to 60% (Figure 3B). In addition, the HFD decreased both male and female biliary excretion of ANIT during the entire time of bile collection, but during the first 15 minutes, the reduction was much greater in female mice than in male mice (Figures 3C and 3D). Both male and female mice had less ANIT biliary accumulation after HFD feeding, but the reduction was more profound in female mice (Figures 3E and 3F).

Liver concentration of ANIT

Livers were harvested after bile and blood collected in plasma elimination and biliary excretion studies, and ANIT in livers were extracted and determined by reverse-phase HPLC described above. Concentrations of ANIT in livers were similar in both genders of CD-fed

mice as well as HFD-fed male mice. Interestingly, levels of ANIT increased 2.5-fold in HFD-fed female mice (Figure 4).

Effects of HFD on ANIT toxicity in female and male mice

The glutathione conjugate of ANIT is a Mrp2 substrate. After efflux into bile, ANIT-glutathione damages biliary epithelial cells, which leads to cholestasis and hepatic injury (Goldfarb *et al.*, 1962; Dietrich *et al.*, 2001). Therefore, reduced expression of Mrp2 will likely result in less biliary excretion of ANIT and subsequently less liver injury.

To test the functional outcome of reduced ANIT biliary excretion, both female and male mice were fed either the CD or HFD for 4 weeks before being challenged with an acute dose of ANIT p.o. administration. Serum parameters that are indicative of hepatic function and biliary integrity (activities of ALT and ALP, and levels of bile acids and bilirubin) (Figure 5A), histological analysis of liver necrosis (Figure 5B) and hepatic neutrophil infiltration (Figures 5C and 5D) were determined to assess liver injury. On the CD, both male and female mice were equally sensitive to ANIT toxicity, as revealed by increased levels of the above parameters. However, the female mice were largely protected from ANIT toxicity whereas the male mice remained sensitive after HFD feeding.

Discussion

The results from the current study showed that Mrp2 mRNA and protein expression was reduced in female mice livers after HFD feeding, while no changes were observed in male mice. Down-regulation of Mrp2 reduced biliary excretion and protected from ANIT toxicity in female mice. These data provide a novel interaction between diet and gender in regulating hepatobiliary excretion and susceptibility to injury.

The effects of HFD on drug transporter expression in the liver have been well characterized. Mrp2 is a major hepatic canalicular efflux transporter responsible for biliary excretion of various conjugated endobiotics and xenobiotics (Lee *et al.*, 1997). The expression and function of Mrp2 is regulated at both transcriptional and post-transcriptional levels. The current study showed that the hepatic mRNA and protein expression and function of Mrp2 was reduced by HFD in female, but not in male C57BL/6 mice. However, the mechanism by which HFD regulates Mrp2 expression and function is not clear. It was previously shown that activation of PPAR α reduces Mrp2 expression (Trauner *et al.*, 1997; Johnson and Klaassen, 2002). HFD is known to activate PPAR α signaling by releasing free fatty acids that are ligands of PPAR α , which may be a mechanism for reduced Mrp2 expression in this study. However, both male and female mice showed induction of PPAR α target genes by HFD (data not shown), indicating that an additional gender-related factor(s) likely participates in the transcriptional suppression of the Mrp2 gene.

It is likely that the effects of HFD on female Mrp2 expression in liver may affect drug disposition in distinct ways depending on the chemical. Consequences of selective Mrp2 reduction may be two-fold. For drugs that rely on Mrp2-mediated biliary clearance, there may be increased systemic and hepatic drug concentrations and toxicity in obese females. For drugs that are toxic to biliary tract epithelial cells, as in the case of ANIT, reduction of Mrp2 protein will help to prevent or at least reduce toxicity in obese females, as in the case of ANIT. In fact, a genetic polymorphism in the human *ABCC2* gene, distinct from the mutations that cause Dubin-Johnson syndrome, is associated with increased susceptibility to NAFLD and disease severity (Silvia *et al.*, 2009).

This study highly suggests that gender differences in drug disposition needs to be considered during preclinical and clinical testing, and effects of dietary factors may also need to be

contemplated. In addition, we have learned in the current study that even when the basal drug disposition of chemicals is similar between males and females, responses to changes in diet, exposure to environmental chemicals, and or during normal aging or various disease states can have gender-dependent effects of pharmacokinetics.

This study strongly supports a concentration threshold for ANIT toxicity to occur. For example, male mice also had reduced ANIT biliary excretion after HFD feeding, but female counterparts exhibited a much lower absolute concentration of ANIT in bile. Yet only HFD-fed females, but not the males, were protected from the ANIT toxicity, indicating that a threshold of ANIT concentration likely exists. Despite the fact that male mice had reduced ANIT excretion into bile after HFD feeding, they were still suffered from ANIT-induced liver injury. This threshold may determine a balance between damage and defense.

Furthermore, this study supports the previously proposed mechanism that hepatic toxicity of ANIT originates from the selective disruption of bile duct integrity, rather than parenchymal cells. In agreement, other studies have shown that bile duct epithelial cells, but not parenchymal cells, were more likely to initiate ANIT toxicity (Hill *et al.*, 1999; Cullen *et al.*, 2010). In the current study, both female and male mice had similar accumulation of ANIT in the liver even after HFD, indicating that the difference in biliary excretion of ANIT accounts for difference in liver injury. The current study, consistent with the study using TR-rats that are deficient in the *Abcc2* gene and protected from ANIT toxicity (Dietrich *et al.*, 2001), suggests that the hepatic toxicity of ANIT likely results from the biliary excretion of GSH-conjugated ANIT and the following disassociation of GSH from ANIT in the bile; high concentrations of ANIT directly injure cholangiocytes, which causes hepatotoxicity.

In summary, the current study clearly showed a gender-specific reduction of Mrp2 expression and function in the liver after high-fat diet feeding in mice. The results of this study suggests that both genders need to be included in considering novel interactions of diet and gender in chemical disposition and toxicity.

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Article Highlights

- High-fat diet (HFD) decreases hepatic Mrp2 expression only in female, but not in male mice
- HFD significantly reduces early-phase biliary ANIT excretion in female mice
- HFD protects female mice against ANIT-induced liver injury

Figure 1. Effect of HFD on hepatic levels of Mrp2 mRNA and protein in male and female C57BL/6J mice

Eight- to twelve-week old C57BL/6 mice were fed a CD or HFD (“western diet”) for 4 weeks. Livers were collected at the end of feeding and hepatic Mrp2 mRNA (panel A) and protein (panel B) levels were measured by real-time qPCR and western blotting (~190 kDa protein), respectively. (C) Representative pictures of immunohistochemistry staining of Abcc2 (Mrp2) in livers of male and female mice fed the CD or the HFD. * $P < 0.05$ (ANIT- vs vehicle-treated mice of the same gender and on same diet fed). # $P < 0.05$ (male compared to female mice receiving the same treatment).

Figure 2. Plasma elimination of ANIT in CD- and HFD-fed male and female mice

Male and female mice were fed a CD or HFD for 4 weeks, followed by 135 $\mu\text{mol/kg}$ dose of ANIT, i.v., Concentrations of ANIT in plasma samples were determined using a validated reverse-phase HPLC method with UV detection as described in the Method section. The plasma clearance parameters are presented in Table 2. * $P < 0.05$ (CD- and HFD-fed groups within same gender).

Figure 3. Bile flow and biliary excretion of ANIT in male and female mice fed the HFD
 Bile was collected from mice for duration of 60 minutes. Concentrations of ANIT in bile were determined using a validated reverse-phase HPLC method with UV detection. (A) Bile flow presented in $\mu\text{l}/\text{min}/\text{kg}$ body weight (BW). (B) Bile flow presented as in $\mu\text{l}/\text{min}/\text{g}$ liver weight (LW). (C) Biliary excretion of ANIT calculated by BW. (D) Biliary excretion of ANIT calculated by LW. (E) Cumulative biliary ANIT excretion presented by BW. (F) Cumulative biliary ANIT excretion presented by LW. * $P < 0.05$ (CD- vs HFD-fed groups within same gender).

Figure 4. Liver concentration of ANIT in male and female mice fed the high-fat diet
Eight- to twelve-week old C57BL/6 mice were fed a high-fat diet ("western diet") or control diet for 4 weeks, followed by an acute administration of ANIT (75 mg/kg). Livers were collected and hepatic concentrations of ANIT were determined by the validated reverse-phase HPLC method with UV detection. *P < 0.05 (CD- vs HFD-fed groups within same gender).

Figure 5. Effect of high-fat diet on acute ANIT toxicity

Eight- to 10-week old C57BL/6 mice were fed a high-fat diet (“western diet”) or control diet for 4 weeks, followed by an acute administration of ANIT (75 mg/kg p.o. gavage). Livers and blood were collected 36 hrs later to determine ANIT toxicity. Panel A: liver histology of CD- or HFD-fed male and female mice with ANIT treatment. Panel B: serum liver function tests (ALT, ALP, bile acids and bilirubin). Panel C: Representative pictures of liver neutrophil staining. Panel D: quantification of liver neutrophil. * $P < 0.05$ (ANIT- vs vehicle-treated mice of the same gender and on same diet fed). # $P < 0.05$ (male compared to female mice receiving the same treatment).

Table 1

Hepatic gene expression in male and female mice under HFD feeding

	MALE		FEMALE	
Gene	CD	HFD	CD	HFD
GCLc	1.0±0.3	0.7±0.0	1.1±0.1	0.9±0.2
HO-1	1.0±0.1	1.1±0.2	1.2±0.4	1.0±0.2
Nqo-1	1.0±0.2	0.8±0.1	1.3±0.1	0.9±0.1
NTCP	1.0±0.1	0.9±0.2	1.2±0.2	0.9±0.1
Mdr2	1.0±0.1	1.3±0.1	0.9±0.2	0.8±0.1
Mrp3	1.0±0.1	0.6±0.1	2.1±0.1	1.3±0.1
Mrp4	1.0±0.1	0.5±0.1	1.0±0.1	0.7±0.1
Oatp1a1	1.0±0.1	1.6±0.1	0.1±0.0	0.1±0.0
Oatp1a4	1.0±0.1	1.0±0.3	1.2±0.1	0.9±0.1

Table 2

Pharmacokinetic parameters ANIT administered intravenously to CD- and HFD-fed male and female mice (135 $\mu\text{mol/kg}$)

	MALE		FEMALE	
	CD	HFD	CD	HFD
Initial body Weight (g)	24.9 $\pm$ 1.0	23.6 $\pm$ 0.9	18.3 $\pm$ 0.8	18.9 $\pm$ 1.1
Body Weight (g) after feeding	25.1 $\pm$ 1.0	26.1 $\pm$ 1.3	19.1 $\pm$ 0.8	21.2 $\pm$ 2.0
Liver Weight (g)	1.2 $\pm$ 0.1	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1
T_{1/2} distr (min)	3.67 $\pm$ 0.61	2.88 $\pm$ 0.42	5.22 $\pm$ 1.64	3.75 $\pm$ 0.56
T_{1/2} el (min)	29.51 $\pm$ 3.24	27.77 $\pm$ 3.62	23.99 $\pm$ 4.86	30.80 $\pm$ 1.97
Vd_{cent} (L/kg)	3.35 $\pm$ 0.45	2.36 $\pm$ 0.32	2.72 $\pm$ 0.32	0.90 $\pm$ 0.09*
Vd_{perif} (L/kg)	7.46 $\pm$ 0.51	8.15 $\pm$ 0.95	8.89 $\pm$ 1.89	2.61 $\pm$ 0.14*
Vd_{app.} (L/kg)	6.34 $\pm$ 0.32	6.46 $\pm$ 0.88	6.06 $\pm$ 1.30	2.11 $\pm$ 0.12*
Cl (L/min/kg)	0.15 $\pm$ 0.01	0.16 $\pm$ 0.02	0.18 $\pm$ 0.02	0.05 $\pm$ 0.004*

Each value represents the mean $\pm$ SE of four to six mice

* Significant difference ($P < 0.05$) from the respective value of the CD group mice.